ON THE RELATIONS OF THE INORGANIC SALTS FOUND IN BLOOD TO THE AUTOMATIC ACTIVITY OF A STRIP OF CARDIAC MUSCLE.

by

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H I S T O R I C A L   R E V I E W.

Haller at the middle of the last century advanced the view that "By the blood, copious, warm and heavy, the sensible flesh of the heart is irritated and excited to action."[I] Haller's ideas held a prominent place in the physiology of the heart until the middle of the present century when the demonstration of the function of the vagus nerve in 1845[2] and the discovery of local ganglia in the walls of the heart in 1848[3] and 1852[4] gave rise to the view that the rhythmic activity of the heart is wholly a question of the control of the nervous mechanism.

In 1853 Carpenter[5] put forth the view that the heart's rhythmic activity is to be regarded "as an expression of the peculiar endowments of its muscular tissue." He regards rhythmic contractility as a "vital" property of cardiac tissue in the same sense that he regards the secreting activity as a vital property of gland cells. "It is not very difficult to apprehend," says

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I. Quoted from Haller ; First Lines of Physiology; p. 40, First American from the third Latin Edition, 1803.

2. E. Weber ; Handwörterbuch d. Physiologie ; III 2, S 42, 1846.

3. C. Ludwig : Ueber die Herznerven des Frosches ; Archiv f. Anatomie, S. 139, 1848.

4. Bidder ; Ueber functionell verschiedene und räumlich getrennte Nervencentra im Froschherze ; Archiv f. Anatomie S 163, 1852.

5. Carpenter ; Human Physiology, 1853.

he, "that the ordinary rhythmical movements of the heart may be due to a simple excess of this motility, which is continually being supplied by the nutritive operations, and is as constantly discharging itself in contractile action."

Brown-Sequard [I] in 1853 repudiates Carpenter's spontaneity theory and says that the heart must have a special exciting cause to set it in activity. He advanced the view that the rhythm of the heart is due to the presence in the blood of carbonic acid, which under some circumstances is capable of acting as a direct excitant of muscular tissue.

As for those writers who maintain that the influence of the nervous system is paramount Brown-Sequard (page 118) pointed out the fact that "they change only the ground of the difficulty. Instead of having to explain why the heart acts rhythmically, they have to explain why the nervous system acts rhythmically on the heart." "They have not explained this rhythmic action of the nervous system", and "they appear not have been aware that this was to be explained

In 1869 Nasse [2] made the important discovery that a solution of .6% sodium chloride would sustain the contractility of the muscles of the frog's leg for a long time. This so-called "indif-

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I. Brown-Sequard, Experimental researches applied to Physiology and Pathology, p. 114, N. Y. 1853.

2. O. Nasse, Pflüger's Archiv, Vol. II, 118.

ferent" solution soon came into general use and proved of great importance in physiological research on the heart.

In 1874 Kronecker and Stirling[1] introduced the perfusion method of studying the nutrition of the heart. They were also first to fill the heart with indifferent saline and study its effects. When the frog's heart was perfused with .6 per cent. sodium chloride solution the contraction quickly decreased to a zero and the heart remained in diastole incapable of contractions even after the strongest stimulus. But such a heart when perfused with blood gave first, fibrillation, then weak contractions and finally contracted as powerfully as in the fresh condition. They showed, too, that a heart could be repeatedly brought to a standstill with sodium chloride solution and again revived by blood. The heart of the terrapin gave the same result except that it took a longer time to bring it about.

They found that small traces of blood in the sodium chloride solution even as little as 0.5 per cent., in a few seconds increased the pulse of the terrapin's heart from 0 to 12-14 mm. of mercury. The results obtained from perfusion of pure sodium chloride solution they ascribe to the removal of the nourishing material contained in the blood in the heart and the succeeding

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1. Kronecker and Stirling, Das characteristische Merkmal der Herzmuskelbewegung. Beit. z. Anat. u Physio . Carl Ludwig gewidmet, p. 173, 1874.

recovery when blood is supplied they ascribe to a renewal of the nutrient fluid.

They say in conclusion that "the heart is almost instantly capable of contractions when it is fed, and it converts the energy which is given to it into work in the most complete and economical way. It ceases to work immediately its food is withdrawn and it does not consume its own substance, but when well nourished and not mistreated, maintains itself for an indefinite time without being consumed."[1]

In 1875 Merunowicz[2] in his now classical research on the frog heart began his discussion with the admission that the "source of automatic stimuli for the frog's heart must remain in the ganglia, so long as the fact holds that the lower two thirds of the ventricle remains quiescent and dies when thrown out of connection with the rest of the heart." But the fact discovered by Bowditch that the isolated apex beats rhythmically if

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I. (Das Herz)" ist fast augenblicklich zur Leistung fähig, sobald es gespeist ist, und verwendet die Spannkräfte, welche ihm zur Verfügung gestellt werden, auf die vollkommenste und sparsamste Weise zur Arbeit. Es stellte seine Leistung gänzlich ein, sobald ihm die Speise entzogen wird, zehrt also nicht vom eigenen Stoffe, erhält sich aber wenn gut ernährt und nicht misshandelt, ohne sich abzounutzen, unbemessene Zeit."

2. Merunowicz, Ueber die chemischen Bedingungen für die Entstehung des Herzschlages, Arbeiten aus der Physiol. Anstalt zu Leipzig, 132, 1875.

Delphinin be added to the nourishing blood led him to examine into the chemical relations of the blood to the heart's rhythm.

He shows that the mere dilution of blood with normal saline, 1 part blood to 4 parts .6% sodium chloride solution, is sufficient to establish a rhythm in the isolated ventricular apex of the frog after a longer or shorter quiescent period (30 to 90 min.) depending on the frequency of the renewal of the solution. A heart that has ceased to beat when filled with serum and sodium chloride gives beats anew when the solution is changed. Delphinin solution added to diluted serum will revive beats after quiescence has been obtained with diluted serum alone, and the converse he finds true also. The long latent period is explained as due to slowness of diffusion rather than to the mechanical shock of preparation.

Merunowicz washed out a heart with serum and sodium chloride for about one hour then placed Stannius's ligatures around different parts of the heart. A ligature 1 mm. below the auriculo-ventricular groove was followed by only a slight temporary disturbance of the rhythm of the isolated ventricle, when a second ligature was placed lower down, the apex continued to beat without interruption. By this means, he proved that the ligature itself was not the cause of the rhythm in the apex filled with saline.

Merunwicz holds that the reason the isolated apex filled

with normal blood does not beat while that filled with blood diluted with sodium chloride solution does, depends upon the fact that the stimulus to the living beating heart does not proceed from the ventricle.

Merunowicz was the first to use a solution of the ash of serum, and an aqueous extract of the evaporated residue of serum. In his experiments he first treated the serum with an excess of alcohol so that his final extract contained only those substances of the serum that are soluble in dilute alcohol. He found that when the extract of the evaporated serum was introduced into a frog's heart that had previously been exhausted by repeated irrigation with .6 per cent sodium chloride solution the heart gave large full beats but at a slower rate than when filled with diluted serum. Solutions of serum ash were not so efficient in reviving the heart beats as serum or as extract of serum, still ash solutions sustained the heart very well and for some time, and "Zeigte sich in der Folge der Schläge die grösste Analogie zwichen dem mit Aschen lösung und mit Blut gefüllten Herzen."

He also demonstrated the physiological effect of sodium carbonate in saline solution and obtained negative results from sodium chloride solution with potassium chloride, a result of which he naturally failed to discover the significance and which was not explained until the work of Ringer, 1883, showed the importance of potassium salts in relation to calcium salts.

By the fruitful results of his experiments Merunowicz was led to the conclusion "that in the region of the apex of the heart as well as in the auricles and the portion of the ventricle immediately adjoining the auriculo-ventricular groove automatic stimuli of the heart's beat are located."[I]

He suggests the view that the origin of contractility in cardiac muscle must depend on the presence of two substances, organic and inorganic. The first is furnished by the muscle in fixed combination, the second by the irrigating surrounding liquid which when it penetrates the muscle converts the organic material into a form which may be used in contractions.

McGuire[2] under Kronecker's direction strove to determine the relative sustaining power of different dilutions of rabbit's blood with saline solution. He found that blood and .6 per cent. saline in the ratio of 1 to 10 was less effective than 1 to 6 but this latter did not produce maximal effects when repeatedly used, under such conditions less dilution, 1 to 2, is better. Blood with an equal volume of saline was not so effective and undiluted blood still less so. That is, "too great a concentration as well as too small a concentration of nourishing liquid is unfavorable" to the heart beat.[3] When the pulse has become very small by the use

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I. "Dass in dem Bereiche der Herzspitze eben so gut wie in den des Vorhofes und der unmittelbar an der Querfurche gelegenen Kammertheile automatische Erreger des Herzschlages enthalten sind."

2. McGuire. Ueber die Speisung des Froschherzens. Arch. f. Anat. u. Physiol. Phys. Abth. 1878, 321.

3. "Es ist also hohe Concentration des Ernährungs materials ebenfalls ungünstig."

of the less diluted blood it may be revived again by more dilute blood or even by pure saline.

He showed also that the lack of free oxygen in nutrient fluid did not destroy contractility, but that the presence of carbon dioxide was positively injurious.

Stienon[I] endeavors to analyze the effect of the constituents of the serum in maintaining the heart's contractions by using preparations of blood with particular ingredients of the serum removed. Neutralized serum, he finds, will not maintain contractility so well as unchanged serum. He attributes this to a loss of sodium carbonate since, if the proper amount of sodium carbonate be returned to the serum it regains its sustaining power. He removed the albumins from serum by heating to the boiling point and then filtering ; by neutralizing the serum, then boiling ; by repeated alcoholic coagulation etc., in each case reducing the extract to a density equivalent to .6 per cent. saline solution. In comparing these albumin free preparations of serum with unmodified serum in their effect on the frog's heart he found that the boiled serum preparations were approximately as effective as serum, only slight differences in the character of the contractions being noted. Serum neutralized then boiled was

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I. Stienon ; Die Betheiligung der einzelnen Stoffe des Serums an der Erzeugung des Herzschlages, Arch. f. Anat. u. Physiol. Phys. Abth. p. 263, 1878.

not so efficient, but when made alkaline with sodium carbonate it was as effective in reviving a quiet heart as the preparations of boiled serum. Alcoholic extracts vary greatly but all show a reviving effect on the heart treated with saline solution but in every case far below that of serum. Alcoholic extracts still containing albumin showed little difference from those free from albumin hence Stienon ascribes the difference in sustaining power between preparations made from alcoholic extracts of serum and preparations made by boiling to a variation in some ingredient other than albumin, especially since the effects from different alcoholic extracts varied greatly among themselves.

Stienon confirms and extends Merunowicz's observation that sodium carbonate added to .6 per cent. sodium chloride solution greatly increases the effectiveness of the saline in sustaining the heart. He finds that this alkali-saline will revive a heart that has become quiet in sodium chloride and keep it beating for hours after each refilling of the heart. Potassium chloride, potassium suplhate, and acid sodium phosphate were non effective, in maintaining contractions. The increased time the heart is sustained by sodium carbonate added to the saline solution can only be explained by the removal of some injurious material, ho

says, or, "dass dasselbe die Umstezung einer schon vorher im Herzen vorhandenen organischen Verbindung fordert."

These conclusions are confirmed and elaborated by the very next article in the same journal. Presumably the two researches were independently pursued, the one from Ludwig's laboratory in Leipzig the other from Strassburg.

Gaule [I] also was attracted by Merunowicz's discovery that sodium carbonate added to saline solution revived a heart exhausted by saline alone.

He confirmed this result and finding that boiled solutions of saline and sodium carbonate were more effective in reviving the heart was led to the conclusion that alkalescence was the efficient property which improved the heart's contractions. He therefore substituted sodium hydrate for the sodium carbonate and obtained the remarkable result that a heart fed with saline made alkaline with sodium hydrate (1 drop NaOH to 500 c.c. .6% saline) "zeichnet mit ihr in ununterbrochener Folge tausend oder mehr Pulse, so dass die Wirksamkeit dieser Lösung nur mit dem vergleichen werden konnte, was Merunowicz von der Mischung von Kochsalz und Blut gesehen hat." The constancy of his results upon hearts under the influence of a purely inorganic diet led Gaule to the con-

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I. Gaule ; Die Leistungen des entbluteten Froschherzens. Du Bois Reymond Archiv. f. Anat. Physiol. (Phys. Abt.) p. 291, 1878.

clusion that "the great amount of work which a heart does under the influence of this inorganic diet can only be produced through the consumption of its own substance," [I] a conclusion which is of fundamental physiological importance and which has led to much fruitful investigation and discussion. This hypothesis, it must be noted was suggested in Merunowicz's paper in 1875.

Gaule found that minute traces of alkali, Na OH 1 to 200000, produce a visible effect and that the effect is most beneficial with 5 milligrams of sodium hydrate to 100 cubic centimeters of .6 per cent saline solution, 1 to 20000. He attempted to show that the alkali was used up in the contracting heart. Believing also that the heart possesses some substances which it gradually used up in contractions he explained the difference in endurance in different hearts to a difference in the amount of this stored material.

To demonstrate this he made an alkali-saline extract of a frog's heart that had been carefully washed and cut into fine pieces and fed this extract to a so-called "cold" frog heart. Now when the "cold" heart was fed on the alkali-saline extract

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I. "Die recht beträchtliche Arbeit, welche ein Herz unter dem Regime dieser anorganischen Diät leistet, kann nur durch Umsetzung seiner eigenen Substanz erzielt werden,"

of heart muscle "then the contractions quickly increased to double or treble the original height." Renewed irrigation no longer recovered this heart after it ceased to beat.

Of the materials which this heart extract might contain Merunowicz had already shown that potassium chloride was inefficient, Gaule found that dextrin, grape sugar and glycogen in dilute solutions were almost indifferent, in strong solutions injurious, but with alkali-saline solutions containing a trace of peptone Gaule obtained a most remarkable result. When this solution was fed to an exhausted or to a weakly beating heart the effect produced "lässt sich nur mit der Wirkung des alkalischen Herzextractes oder der blutigen Kochsalzlösung vergleichen und ist nicht geringeals diese." Gaule's experiments indicate that the efficiency of alkali-saline is due, in part at least, to its combining with carbon dioxide set free during the activity of the heart muscle. This fact is emphasized later by Martius in 1882.

Gaule's results with peptone are most interesting but unfortunately later observers who have used peptone have totally failed to verify his observations. The peptone effects he obtained must, therefore, be held in question until other positive results are brought forward to support them.

In 1881 Aubert [I] clamped off the apex of the frog's heart by Bernstein's method and verified Bernstein's and Bowditch's results. He kept frogs from 1 to 41 days and found that the isolated apex was still quiet but irritable, responding by a single contraction to a single stimulus. The complete destruction of muscle continuity was in his experiments afterward proven by histological examination...

He divided the ventricle into zigzag portions by successive clampings leaving a small unclamped bit of muscle on alternate sides of the heart. In these experiments these portions of the ventricle always contracted following a sequence set by the basal portion.

He also verified the observation of Merunowicz that a quiet isolated apex filled with blood may be made to beat by filling it with .6 per cent. sodium chloride solution.

But the peculiar discovery of Aubert, and one of great importance in the explanation of the sequence of the beat of the parts of the heart, according to the myogonic view of the cause of the rhythm, is the fact that an isolated apex made to beat rhythmically with saline solution is brought into a quiescent state again when irrigated with pure serum. He repeated this

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I. Aubert :
Untersuchung über die Irritabilität und Rhythmicität des nervenhaltigen und nervenlosen Froschherzens : Pflüger's Archiv., Vol. XXIV. p. 357, 1881.

observation back and forth several times on the same heart. Aubert suggests that this fact implies the presence in the blood of mammals as well as frogs, of some constituent or condition, albumin, concentration or the like, in the absence of which the heart apex beat. In his own words "esm giebt Bedingungen unter denen der nervenlose Herzmuskel rhythmisch pulsirt, und Bedingungen, unter denen er, ohne pulsationsunfähig zuzein, nicht pulsirt." His theory is that there are conditions of inhibition and conditions of activity for the heart muscle as well as for the nerves. In this view Aubert thought to harmonize the law of Merunowicz that the apex as well as the auricle and the base of the ventricle is automatic, and the opposing law of Bernstein that under normal physiological conditions no automatic contractions arise in the ventricle.

Martius [I] 1882, under Kronecker's direction, was the next to take up the question of the nutrition of the frog's heart. He holds that the heart muscle, like the steam engine, can work only when supplied by energy giving material from without, and that the nourishing material is supplied from the blood. "So long as there are remnants of blood for the assimilation of the isolated heart muscle it works" provided a sufficient stimulus is

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I. Martius. Die Erschöpfung und Ernährung des Froschherzens : Archiv f. Anat. u. Physiol. Phys. Abth 1882 p. 542. 1882.

supplied. The conclusion of Gaule that the heart muscle works at the expense of its own material is met by supposing that Gaule experimented with imperfectly washed hearts that there were still remnants of blood in the spaces between the cardiac fibres, and that these remnants were the source of the energy-giving material rather than the muscle substance itself. In this way Martius explains away Gaule's beneficial effects of peptone, that is, he holds that Gaule was deceived by the imperfect removal of blood in the washing of his hearts. The fact that a heart exhausted by repeated changes of .6 % saline solution is revived and gives good strong beats when fed with saline made alkaline with sodium carbonate (Merunowicz, Stienon), or sodium hydrate (Gaule) is to be explained by the setting free of nutrient material in the intercellular spaces. This material is liberated by the alkali taking up the carbon dioxide and is at once used in the work of the heart. A heart that has beat to a standstill on repeated changes of saline, then on repeated changes of alkali-saline may be considered as truly exhausted of extraneous nutrient material, says Martius. On such a heart one may really prove whether any particular ingredient of the blood is nutrient or not. On alkali-saline "exhausted" hearts Martius obtained totally negative results with peptone solutions made in alkali saline. Syntonin, egg-albumen, casein of milk

and glycogen were also negative.

The only solutions he found that would restore an exhausted heart were blood, serum, (diluted with saline 1:3) and lymph. He therefore, concludes first, in opposition to Gaule, that the frog heart can never work at the expense of its own material, and supports Kronecker's view that the heart works only when supplied with a nutritive material on which it can draw for energy, and that it ceases to work when the energy-giving material is withdrawn ; and second, only fluids containing serum albumin (blood, serum, Lymph) are capable of nourishing the heart, that is, of supplying energy for the production of work.

Since 1880 Ringer has actively pursued investigations towards the discovery of the part taken by the inorganic salts of blood in physiological processes in general. It is to him more than to any other that physiologists are indebted for our present knowledge of the importance of potassium and especially of calcium salts in the contractions of the heart. In 1883 Ringer[1] showed that minute quantities of calcium salts in normal saline, 1 in 20,000, will restore contractions in a frog's heart after it has become quiet in saline alone. He found that calcium produces

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1. Ringer; Further contributions regarding the influence of the different constituents (inorganic) of the blood on the contractions of the heart, Journal of Physiology, Vol. IV. 1883, p. 29. See also articles by the same author in the Journal of Physiology Vols. IV, 370, V. 352, VI. 154, VII. 291, VIII. 20, and 288. XIV.125, XVI. 1, and XXII p. XIV.

prolongation of dilation and even persistent spasm. If, however, potassium chloride be added to calcium in saline solution the injurious effects of the lime salts are completely removed, and the combination makes a very good circulating fluid. Since the heart's contractility cannot be sustained by sodium salts alone or by sodium and potassium salts. Ringer concludes "that a lime salt is necessary for the maintenance of muscular contractility". But while a calcium salt is necessary he also holds that it must be antagonized by a potassium salt.

Von Ott [1] in 1883 found that peptone solutions would not revive an isolated frog's heart after it ceased to beat on sodium chloride solution, but if the peptone was first brought into contact with the living tissue of the intestine or the stomach it would then make the frog's heart beat. He attributed this result to the formation of serum albumin i. e. an albuminous body capable of nourishing the frog's heart.

Kronecker and Popoff [2] and Brinck and Kronecker [3] in 1887 confirm von Ott's observations. In fact they consider the ability of a solution to revive beats in a frog's heart as proof positive of the presence of serum albumin. The latter observers

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1 von Ott, Ueber die Bildung von Serum albumin in Magen etc. Archiv f. Anat. u. Physiol. Physiol., Abth. p. 1, 1883.
2. Kronecker and Popoff, Ueber die Bildung von Serum-albumin im Darmcanale, Archiv. f. Anat. u. Physiol. Physiol. Abth. p.345, 1887
3. Brinck and Kronecker, Ueber synthetische Wirkung lebender Zelle Archiv. f.Anat.u.physiol. Physiol. Abth. p. 347, 1887.

say that this physiological test is more [illegible] for serum albumin than physical or chemical tests. This assumption apparently rests on a firm confidence in Martius's conclusion that only fluids contain serum albumin can sustain the heart in rhythmic contractions for long periods. They all apparently overlooked the investigations of Ringer who questioned the truth of the hypothesis on which von Ott's and Kronecker's work is founded.

In 1892 Heffter[1] in a series of experiments on the nutrition of the frog's heart observed that blood from the horse, ox and pig when diluted with saline all have good sustaining power. Serum on the other hand will not call forth as much work from the heart. Free oxyhaemoglobin will not support the heart, neither will washed blood corpuscles in physiological salt solution. He finds however, that washed blood corpuscles suspended in a solution of egg albumin or in a two per cent. solution of gum arabic neutralized with sodium carbonate will support the heart in splendid acttivity. "The gum arabic solution appears even to be a better medium than egg albumin solution" therefore Heffter concludes that a nutritive fluid must have certain physical properties such as the blood or serum possesses and that the blood corpuscles are to be considered as the primary factor in supplying the energy for

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1. Heffter, Ueber die Ernährung des arbeitenden Froschherzens, Archiv. fur Experimentelle Path. u. Pharm. : Vol. 29, 1892, p. 41.

the heart's contractions.

Howell and Cook [1] in attempting to thoroughly exhaust frog's hearts found "that after exhausting a heart first with normal saline, and next with Martius' solution, the use of Ringer's solution would again revive it and keep it beating with vigor and regularity" for as much as thirty hours. They consider it improbable "that even a minute trace of serum albumin should have been left in the circulating liquid." They showed too, that extracts of the inorganic salts of serum, milk, and gastric juice in the concentration in which they exist in these liquids will revive a frog's heart after it has ceased to beat in normal saline. A heart irrigated with solutions of these salts will beat for several hours, and the beats revived are normal in character. These observers suggest that the recovery brought about by peptone which is first submitted to the action of gastric juice in the stomach is not necessarily due to the formation of serum albumin as von Ott and Kronecker assume, but that it is more probably due to the action of the inorganic salts added to the solution while it is in the stomach.

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1. Howell and Cook, Action of the inorganic salts of serum, milk, and gastric juice, etc., upon the isolated working heart; Journal of Physiology, Vol. XIV. p. 198, 1893.

They conclude in support of Gaule's view that "the heart is able to contract at the expense of its own substance when properly stimulated" and, therefore, that Martius' deduction as to the importance of serum albumin must be abandoned. Ringer's views concerning the role of potassium and calcium salts in maintaining the rhythmic activity of the heart are supported by these investigators. The beneficial effects of gum arabic in maintaining the heart's contraction attributed by Heffter to the viscosity of the solution these authors attribute to the fact that gum arabic is a compound of arabin and calcium and potassium salts.

In 1895 Locke [1] found that a sodium salt of the acids found in gum arabic when added to alkali saline did not sustain the heart But when calcium and potassium chlorides were added in the necessary amounts its activity recommenced and there was also good recovery of its absolute force.

White[2] in 1896 again discusses the nutrition of the heart from Kronecker's point of view. He holds that many observers especially those who have used the William's cannula have not secured perfectly washed out hearts and that their conclusions are therefore vitiated . White by alternately washing the

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1. Locke, Towards the ideal artificial circulating fluid for the isolated frog's heart ; Journal of Physiology, Vol. XVIII, p. 332, 1895.
2. White; On the nutrition of the frog's heart, Journal of Physiology, Vol. XIX. p. 344, 1896.

frog's heart with saline and with Ringer's solution, brought it into a state in which neither Ringer's solution nor a strong stimulus would produce contractions. Blood would again restore retractions in such a heart. White admits that the salts of sodium, calcium and potassium are "necessary to the chemical changes which occur during contraction". He says that "it is only by such a fluid as Ringer's, which contains the salts of the blood in natural proportions, that all the nutrient material in the form of blood residue has been removed. When we wash out with an 'indifferent' fluid (saline solution), the albuminous material is only very gradually removed, but the salts are easily and consequently very soon completely washed away". Since both salts and albumin are necessary to the heart's activity" therefore, beats continue on Ringer's fluid until the last trace of albuminous material is removed from the capillary clefts. When a heart is thus exhausted "it can only be restored by feeding it with a true nutrient fluid (such as blood, serum, and lymph) containing booth serum albumen and salts, the former being most essential, while the latter play a secondary but necessary part."

METHODS AND APPARATUS.

In 188[illegible] Gaskell demonstrated that a strip of the apex of the terrapin's ventricle would give automatic rhythmic contractions when suspended in a moist chamber and left to itself. This simple experiment offers a method for the study of rhythmic muscular tissue as such. An intricate net work of nerve fibres interlaces among the muscle cells of the apex of the ventricle and, according to Berkley,[1] an occasional isolated nerve cell is found in even the apex in the frog and the mouse, still it can scarcely be supposed that a stimulus that acts to produce rhythmic contractions in the isolated strip of the ventricle acts on the terminal nerve elements to the exclusion of the muscle cells. However this may be, whether the automatism resides in the muscle or nerve, the phenomena associated with the rhythm of an isolated strip of muscle seem to admit of a more definite study than the phenomena associated with the activity of the completely organized heart itself.

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1 Berkley; The intrinsic nerve supply of [illegible]; Johns Hopkins Hospital Reports, IV, 248, 189[illegible].

In a preliminary [illegible], the apex muscle strip of the terrapin's ventricle [illegible] remained alive and gave off rhythmic contractions for [illegible] days. This preliminary test fully demonstrated the validity of the method, and I have, therefore, adopted this method for the study of the inorganic constituents of blood in their relation to the activity of cardiac muscle. The following pages will, I hope, fully demonstrate the directness, reliability and simplicity of the method.

My plan of procedure has been in brief, to suspend a slender strip of the apex of the ventricle in a moist chamber, attach it to a recording lever under a definite tension, submit it to an artificial fluid by immersing it in the solution or by moistening it in air, and to record the resulting phenomena by the lever and by direct observation. The extraordinarily long life of the muscle under such conditions permits a series of experimental changes affecting each strip. By this method one has the advantage of placing several strips from the same heart simultaneously in different solutions.

APPARATUS :

The requirements of this method are very simple and no intricate apparatus is needed. It is necessary only that the muscle be protected from evaporation [illegible] with a delicately poised recording lever. [illegible]

ed has the additional advantage of all the desired mechanical conveniences in adjustment.

The muscle is suspended in a thin walled glass tube 14 cm. in length and of a diameter of 1.5 cm. to 3. cm. according to the experiment. This tube is held in a vertical position by an ordinary burette clamp and stand. The tube is closed at the bottom by a rubber stopper containing a small glass cannula. The lower end of the cannula is closed by a short rubber tube and pinch-cock while its upper end opens at the surface of the stopper in such a way as to permit the filling or the complete emptying of the heart tube without disturbing the heart strip. One side of the upper or inner end of the cannula is drawn out and bent into a small hook for the attachment of the lower end of the muscle strip. The upper end of the heart tube is ground flat and is covered with oiled paper. Or, in the tube used for air experiments, the top is constricted into a small neck with funnel-shaped outer end. The neck and funnel can be filled with a drop of water which offers no resistance to the movement of the thread passing through it, and at the same time prevents evaporation from the heart tube.

Either silk ligatures were tied around each end of a muscle strip and one thread closely looped over the glass hook at the top of the stopper cannula and the other carried to a lever above,

or [illegible] S-[illegible] hooks were [illegible] of the muscle strip. The hooks [illegible] for [illegible] comparatively large ventricular strips.

A light straw lever was used in these experiments. The lever was balanced on a steel rod 1 [illegible] diameter by means of a glass hub fitted tightly into a hole burned through the straw. This tube was about 1 inch long and its ends were [illegible] so that the weight of the lever rested wholly on a very small bearing surface. This device reduced the friction of the lever on the steel wire support to a minimum. The fulcrum of the lever was placed between the attachment of the muscle and the writing point, thus converting the downward pull of the lever into an upward stroke on the recording cylinder. The lever carried a five gram weight near the supporting glass hub so adjusted that the total weight on the muscle was approximately one gram.

The axis of the lever was supported above the heart tube by means of a rod and [illegible] so arranged that the lever could be readily adjusted in any plane. The horizontal adjustment was found extremely convenient since by it the recording lever could be taken from the recording surface or [illegible] at any moment without in the least disturbing [illegible] attached to the same stand.

A slow drum was [illegible]

given by the terrapin's ventricle strip, especially when the experiment continued through a series of hours including, perhaps, two or three consecutive nights. The particular drum used for most of these experiments had a circumference of 47 centimeters and made one revolution in 11 hours.

When parallel experiments were made, as was always the case, except in one or two experiments, two and three heart tubes with levers were attached to the same stand, the levers writing one above the other.

The artificial solutions used in these experiments were in every case made in water distilled from glass. The absolute necessity of this precaution in any series of experiments involving the effects of the inorganic salts of blood on living animal tissue was first demonstrated by Locke[1], and more recently has been confirmed by Ringer.[2]

These investigators showed that minute traces of certain of the heavy metals, such as copper, dissolved from the copper receiving tanks so often used, may completely obscure the effects of the particular salt experimented with. The water used in my experiments was prepared in part by the method devised by Jones

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[1] Locke; On a supposed [illegible] certain animal organisms; Journal of Physiology, 18, 319, 1895.

2 Ringer; The action of distilled water on Tubifex; Journal of Physiology, 22, XIV. Dec. [illegible], 1897.

and Mackay [1] except that potassium bichromate was substituted for permanganate of potassium in the first flask, according to a recent suggestion of the authors, and that I used a Jena glass condenser tube instead of a block tin tube used by them. More recently the water used was purified by making ordinary laboratory distilled water slightly alkaline with sodium hydroxide and re-distilling in glass.

The sodium and potassium salts used were purified by repeated recrystalization of "chemically pure" salts. Solutions were made by dissolving a weighed quantity of the dry salt in a measured quantity of distilled water. Stock solutions of one per cent. strength were prepared from all salts, except, of course, sodium chloride, and solutions for immediate use were made up from these stocks as needed. In the case of the deliquescent calcium chloride a solution was prepared of approximately the strength required and the amount of calcium in this solution quantitatively determined by the usual gravimetric method of precipitation as calcium oxalate and reducing to calcium oxide. From this general solution a one per cent. stock was prepared.

In the details of experiments the proportions of the various salts used in any given artificial solution are expressed in terms of percentage.

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1 Jones and Mackay, Eine wirksame und bequeme Methode Wasser zu reinigen; Zeitschrift f. physikalische Chemie, XXII, 2, 1897.

INTRODUCTORY.

It is the intention in this paper to investigate one of the conditions of automatism in the tissue of the heart rather than to discuss the old question of whether the automatism of the heart resides primarily in the muscular tissue or in the nervous mechanism. Brown-Sequard[1] pointed out more than fifty years ago that the more fundamental question regarding the heart's activity is not which tissue is automatic but why or under what conditions either tissue shows automatism. I emphasize the idea of conditions of automatism with intention, for while one may justify himself in setting set forth hypotheses for the explanation of results obtained under the multiple conditions of experimentation, still one must fully recognize the impossibility of reaching a decisive determination of a question which at the present time involves so many necessarily unknown factors.

The influence of the blood on the heart's contraction must necessarily be considered both from the physical and from the chemical point of view. The characteristics of the blood are isotonicity with [illegible], a certain degree of viscosity, and a complex chemical composition.

Since the experiments of [illegible] in 1869 on the effects of different strengths of sodium chloride solution on the irritabil-

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1 Brown-Sequard, Experimental researches applied to physiology and pathology, p. 114, N. Y. 1853.

ity of the gastrocnemius of [illegible] tissue has been recognized by all physiologists as the first requisite of any artificial solution designed to maintain the activity of living tissue. In these experiments the effort has been made to keep this factor constant by using solutions as nearly isotonic as possible, except in a very few special cases.

Certain investigators, notably Heffter[1], have insisted upon the importance of a degree of viscosity in an artificial circulating fluid. Others have disproved, or at least have thrown grave doubts on the validity of this conclusion, and until further evidence is brought forth we may safely ignore this property in any investigation on the chemical constitution of the blood in its relation to tissue activity.

As a basis for the preparation of artificial solutions chemical analyses of blood or serum as found in the literature are in many respects incomplete. The most common defect is the lack of information as to the exact forms of the salts present and the proportions in which they exist as free salts and as salts in combination with organic components.

Then, too, such salts as are found only in minute quantities are not quantitatively determined, although from a physiological point of view they may be of very great importance.

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[1] Heffter, Ueber die Ernährung des arbeitenden Froschherzens, Archiv f. Exp. Path. u. Pharm., [illegible], [illegible], [illegible].

The most abundant salts of blood are those of sodium, potassium, calcium and magnesium. The historical facts regarding these salts in their relation to the activity of the heart are set forth at some length in the review of the literature of the subject at the beginning of this paper. In experiments up to the present, however, the heart of the frog has been used almost exclusively for experiments of this kind.

The experiments described in this paper were confined to the terrapin and were performed exclusively on strips of muscle cut from the apex of the ventricle. The strips were taken either from ventricles filled with blood or from ventricles freed of blood by irrigation with normal saline solution. In the latter case the heart was isolated and irrigated through an inflow cannula inserted into the left vena cava and an outflow cannula in one of the aortic branches. The coronary arteries vary much in their origin in the terrapin used but care was always taken to establish as good a coronary circulation as possible by cutting one of the coronary veins.

From two to four slender strips were prepared from each heart by first cutting off the lower two-thirds and then splitting this into the requisite number of pieces. The usual strip was approximately one inch long and weighed two to three tenths of a gram. In all over fifty different hearts were used giving from two to four strips each and each strip was submitted to from five to ten

di[illegible] in the experi[illegible] of each [illegible] was observed for periods varying from one to sever-al hours. The average duration of the period of action of a single strip was from forty to fifty hours although many exceeded seventy-two hours and some reached ninety-six to [illegible]-eight hours.

It will readily be seen that the total time of suspension and the number and character of changes preceding any particular test are most important factors in determining the results of that test, and that the unifying of the results of the entire series of experiments is correspondingly difficult.

SERUM.

Before discussing the relation of solutions of the inorganic blood salts to the action of a ventricular strip let us first consider the behavior of such a strip immersed in its own serum. If an apex strip cut from the heart of a terrapin, Chrysemys picta, is suspended in a heart tube and covered with normal terrapin's serum, absolutely no rhythmical contractions are developed. If the preparation is not quickly made and is allowed to evaporate slightly during the process, a few single contractions may occur when the strip is first suspended. The number of such contractions does not exceed ten or fifteen and they occur during the thirty to sixty minutes following suspension.

In five experiments strips were kept in serum for from twenty-one to one hundred hours. After [illegible] hours and [illegible]

sions of contraction in [illegible] air. [illegible] in serum the [illegible] are frequent than [illegible], but they do not on an average exceed one an hour in strips kept for a very long time.

One strip, number 47d, left undisturbed for seventy-six hours in serum, gave during that time eighty-two single contractions, seventy-five of these occurred during the last half of the time. Meanwhile bacteria appeared in the serum, this was therefore drawn off and the strip left in air, though still moist with serum. The muscle at once began to contract with full normal contractions and continued to do so for six hours, when the recording drum stopped and further record was lost. Another strip was kept in serum for ninety-six hours and gave only forty contractions during about one-half of the time. The record was lost at intervals during the experiment. At the end of ninety-six hours this strip was quiet in a relaxed state but when changed to .6 per cent. sodium chloride solution it immediately gave a series of rhythmical contractions. These contractions were more irregular in rate than is usual in a sodium chloride series such as will be described later, they were at first 1.[illegible] cm. to 1.[illegible] cm. high and gradually decreased to zero within one hour and a half . After one hundred hours [illegible] of Ringer's solution produced a strong increase in tone [illegible]

ing that the muscle was still alive and irritable.

From these experiments it will be seen that serum preserves the heart strip in good condition for contraction for a very long time, but that it does not supply the necessary conditions for the development of rhythmic contractions of ventricular muscle.

It is well known that an isolated apex of the frog's ventricle will not give contractions when the heart is filled with its own blood, a fact made use of since its discovery by Bernstein in 1876 to support the views that the contractions of the heart are nervous in origin. It is significant, therefore, in light of what I shall present later, that the ventricular strip agrees in its reaction to serum with the apex of the frog's heart when isolated by Bernstein's method.

MODIFIED SERUM.

In 1875 Merunowicz[1] found that blood of the sheep or rabbit is most favorable to the development of good contractions in the frog's heart when diluted with .6% sodium chloride solution in the ratio of one part of blood to four parts saline. McGuire[2] in 1878 investigated the relative proportions of rabbits' serum and .6% saline in reference to their sustaining

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1 Merunowicz; Ueber die chemischen Bedingungen für die Entstehung des Herzschlages, Arbeiten aus der physiol. Anstalt zu Leipzig, 132, 1875.

2 McGuire: Arch. f. Anat. u. Physiol., 321, 1878.

power of the frog's heart. [illegible] mixture of serum and saline in the ratio of [illegible] does not produce maximal contractions; that 1 to 2 is [illegible] concentration of serum is "unfavorable." At the present day following [illegible] and similar results obtained by other observers it is customary to dilute rabbits or other mammalian blood with sodium chloride solution when it is to be used for artificial circulation experiments, without inquiring why the dilution improves the blood in its adaptability to the production of good rhythmic contractions. I have repeated the experiments of Kronecker and McGuire on the apex strip and have other experimental evidence which I hope will show to what dilute serum owes its efficiency. It has already been shown that terrapin's serum in its normal concentration and composition as well as mammalian serum is inefficient in developing rhythmic contractions in a heart strip.

Experiment number 24, December [illegible], 1897.

A strip from a perfectly fresh heart was suspended in fresh serum kept over from the preceding experiment. During the succeeding four hours, twelve contractions were developed. These contractions varied in amplitude from 0.1 cm. to 1. cm. and occurred at very irregular intervals. The [illegible] contractions were submaximal. Sodium chloride .6 per cent. was added after four hours at successive intervals [illegible] ten minutes. After twelve minutes

full strong contractions began which were at first 1. cm high but increased to 1.2 cm. in two or three minutes, then slowly decreased to .8 cm. in the two hours following. The rate was quite irregular in this series and the contractions were in groups of varying rates. Ten minutes after the series began one group showed at the rate of 28 per minute. As the height decreased the rate became slower and slower. After two hours contractions came only at long intervals and so continued for 24 hours.

Experiment number 30^b.

An apex strip was immersed in .64 per cent. sodium chloride solution until the contractions resulting decreased from 1.15 cm. to .02 cm. in height. The saline was then drawn off and normal serum introduced, 4 hours and 5 minutes after suspension. The strip immediately exhibited uncoordinated contractions, and fibrillation. At the same time the strip shortened in consequence of an increase in tone. In fifteen minutes the fibrillation disappeared and regular contractions of large amplitude began and the tone passed gradually away. The rate of contraction became slower as the contractions became more nearly normal in height until in fifty minutes the strip remained quiet in a relaxed condition. The muscle was apparently in a state comparable to that of a fresh strip at rest in a serum bath.

After forty minutes quiet the serum was diluted with .6%

sodium chloride in the ratio of 1 part serum to 2 parts sodium chlride. There was no change for [illegible] minutes, the contractions began 1.7 cm. in height and with a very irregular rate. The contractions gradually decreased in rate and ceased in forty minutes. Six hours and twenty-five minutes from the time the serum was first used the serum-saline was further diluted to the proportion of 1 part serum to 6 parts sodium chloride. In ten minutes a perfectly regular series of contractions began and continued for four hours. The rate during this time slowly decreased from 2 to 1.3 per minute but was otherwise perfectly regular. The amplitude was exceptionally great, 1.7 cm. Between 10 hours, 25 minutes and 16 hours 35 minutes after the serum was first introduced the strip remained for the most part quiet in diastolic state, only occasionally giving a contraction. That the above heart strip was in good condition all the time was shown by the beautiful series procuded when later the strip was transferred to .6 % sodium chloride. Attention may here be called to the fact that the serum saline series of regular contractions extends over a time as long or longer than that of many experiments on the frog's heart noted in literature, and that here as in most of the experiments in this research, the experiment was continued until the after effects were fully determined. For this particular strip I have a continuous record through successive experimental conditions for seventy-three consecutive hours.

From the above description it may be noted that automatic rhythmic contractions are developed in the ventricular strip by submitting it directly to a bath of serum diluted with a large amount of physiological saline. Also that beats are developed in a strip saturated with sodium chloride and surrounded by serum. That is, it may be assumed that beats occur during that early period in the process of diffusion when the salts of the serum diffusing into the muscle mass may be assumed to be very much diluted.

It must also be noted that a strip that is quiet in normal serum may be made to beat with perfect rhythm and complete and normal amplitude although for a variable time by increasing the percentage of calcium salts in the serum.

Further, serum will revive activity in a heart strip after it has been thrown into a state of strong tone accompanied by fibrillation in consequence of the action of other solutions such as sodium chloride solution containing calcium chloride, or a Ringer's solution used after sodium chloride. The exact type of recovery depends upon the degree of general exhaustion of the strip, i. e. upon its total activity previous to suspension, and upon the length of time that the strip has been in fibrillation. When such a fibrillating strip is changed to serum it immediately begins to increase in activity, giving stronger and more rapid fibrillary contractions. After a longer or shorter period depending on the above mentioned conditions the strip gradually con

es fibrillation, partially relaxes and then begins contractions that are normal in type but irregular in rate and not frequent. The contractions obtained from strips that are in a more nearly exhausted state are small at first and gradually increase in height during several hours. Almost invariably, however, the contractions in serum become more and more infrequent and practically cease after five to fifteen hours. This condition is again comparable to that of a fresh strip immersed in serum, except that fatigued strips, it must be remembered, have been under experimentation for several hours and have already expended a great amount of energy in contractions.

Finally, a muscle by proper manipulation may be made to use up its available supply of energy-giving material. A muscular strip beating in a solution of inorganic salts may ultimately gradually decrease in the amplitude of its contractions and pass into a state from which it never recovers its original amplitude. When in this state it is only slightly revived by serum. This is a state of true exhaustion which will be discussed in more detail later.

If such an exhausted strip be immersed in pure serum it will give a series of feeble contractions. These feeble contractions are often of quite a rapid rate, but do not even approximate the height of contraction given by the strip before exhaustion. They quickly dis[illegible]

hausted strip revives quite [illegible] contractions that persist a relatively long time. By [illegible] of exhausted strip with the proper combination of [illegible] recovery in rate is produced but never quite as much as with serum.

Serum will revive a strip from the quiescent state following exposure to the action of solutions of sodium chloride ; or sodium chloride plus potassium chloride such as will be described later ; from a state of tone and fibrillation caused by the use of Ringer's solution containing excess of calcium salts after sodium chloride quiescence ; in fact serum will apparently revive a heart strip from almost any unfavorable condition brought about by isotonic solutions of the inorganic salts found in the blood except possibly that condition of true exhaustion which has been briefly mentioned above and is described more fully in the chapter on exhaustion.

SODIUM CHLORIDE.

Sodium Chloride is the most abundant salt in the blood. Its solution in amounts isotonic with the blood has, therefore, for nearly thirty years been in constant use as the so-called indifferent or normal physiological saline. It is the only simple substance so far as now known that [illegible] used in an artificial circulation medium to preserve the isotonicity with the tissues, and isotonicity seems to be a necessary factor in [illegible] experiment on artificial solutions. I have, therefore, taken up first, in my experiments the effects of solutions of sodium chloride on the

develop[illegible]

apex [illegible].

It was [illegible], [illegible] apex of the [illegible], [illegible] latent period, [illegible], [illegible] with .6 per cent. [illegible] chloride [illegible] with normal [illegible], [illegible] .6 per cent. sodium chloride. Later Aubert, [illegible] showed [illegible] not only [illegible] [illegible] chloride [illegible] that it [illegible] blood; [illegible] further, [illegible] rhythmically or remain quiet according as [illegible] fed with sodium chloride solution or with blood. Aubert's [illegible] least demonstrated [illegible] "indifferent" to living cardiac tissue [illegible] serum.

I [illegible] show what [illegible] place when an apex [illegible] normal sodium chloride solution. [illegible] 0.6 per cent. sodium chloride [illegible]. [illegible] cool [illegible]. [illegible] chloride solution it begins to [illegible] after a certain latent period. [illegible], [illegible] trations [illegible]

[illegible]

T A B L E I.

Table showing the great diversity of length of the latent period, of rate, and of maximal height of contractions produced by immersing a fresh ventricular strip of the terrapin in normal sodium chloride. The height is given as the actual shortening of the strip in contraction.

Number of the Experiment	Latent period before contractions begin.	Maximal height of contractions	Rate when the rhythm first becomes regular, together with the maximal rate in some examples.
19 b.	11 min.	1.3 cm.	12 to 14 per min.
28 b.	1 hr.15 "	.15 cm.	3 " "
29 b.	1 " 30 "	.2 "	6 increasing to 10per min
31 a.	2 " 35 "	.32 "	2.2 " to 7 per min.
32 a.	40 "	.9 "	6 " " 9 " "
39 c.	25 "	.7 "	8.5 " " 10 " "
40 c.	1 " 50 "	.5 "	4 " " 8 " "
46 c.	2 " 3 "	.6 "	4 per min.

[illegible] , [illegible] . [illegible] . In some experiments, however, it was found to be [illegible] though the solution was often renewed.

The height of the contractions varies much in different series, although in general it may be said that the longer the latent period the shorter the contractions when they do begin, and the shorter the time they are continued. I cannot at present give any evidence showing any quantitative relation of the rate to the latent period, etc. It is to be noted that a sodium chloride series when once established goes through a regular series of changes in the rate and in the height of the contractions. The rate is almost always slow and irregular at the initiation of the series, becomes regular after 0-20 minutes, then slightly increases in frequency while decreasing in amplitude until the contractions are reduced to the fraction of a millimeter in height. The height of the contractions in the series is at first gradual, quickly increases to a maximum, and then very gradually decreases to a millimeter or less into what has been designated as the state of "saline contraction". If the muscle is left undisturbed in the solution while it is giving the series of contractions the series decreases in height with beautiful regularity. But if during the series the solution on

the heart strip be renewed [illegible] generally causes a slight increase in the height of the contractions and is almost always followed by an increase in rate. When the strip has become quiet in sodium chloride solution, renewal of the solution has only a very slight effect or else no effect at all in recovering contractions in the strip. If there is any recovery it is never more than a minute fraction of the original amplitude.

With saline made slightly alkaline with sodium carbonate I have never obtained a recovery of more than a small fraction of the original amplitude of the beat. This fact is noteworthy in comparison with results obtained on the saline "exhausted" frog's heart by Gaule, Stienon, Martius, and White. According to my observation it would seem that the heart muscle of the terrapin treated with sodium chloride alone passes into a state from which it cannot be revived by alkaline sodium chloride solution. Although in consequence of sodium chloride treatment it gives smaller and smaller beats and finally remains perfectly quiet in the relaxed state, still, as will be shown, the muscle strip may be thrown into most powerful rhythmic contractions at any moment if only the proper inorganic salts be added to the saline solution. Then the term [illegible] therefore, [illegible] with the saline [illegible] applies only to the pe[illegible] condition of [illegible]

treatment with [illegible], [illegible] be designated more accurately [illegible] as sodium chloride pause.

The amplitude of the contractions [illegible] chloride series is no criterion as [illegible] contractions which the strip will give under [illegible] conditions, i. e. other solutions of the inorganic [illegible] blood. Some of the strips of my experiments which I had considered as from animals in bad condition [illegible] gave poor sodium chloride series, afterwards when subjected to a bath of other inorganic salts gave the most unexpected contractions as regards amplitude. Strips from hearts which have previously been thoroughly irrigated with sodium chloride solution give only minute beats or none at all when suspended in a sodium chloride bath. They react in fact like the normal strip suspended in saline at the time that the saline series is nearly at an end, the exact similarity depending somewhat on the thoroughness of the irrigation. These strips, also, are [illegible], as an experiment to be described later ([illegible], 73) [illegible].

The great diversity [illegible] exhibited by ventricular strips [illegible] at [illegible] because these are the [illegible] the [illegible]. As a matter of fact [illegible]

expected [illegible] the other [illegible] in [illegible]. [illegible] this paper [illegible] difference.

If the strength of the sodium chloride to which the muscle strip is subjected is varied certain interesting phenomena are noticed. In two sets of experiments of three strips each the strips in hypertonic solutions of sodium chloride began rhythmic contractions after a very short latent period, a latent period shorter than was ever obtained in the fresh strips in isotonic solutions. The strip in hypotonic solutions, on the other hand, were not constant in their behavior. One had an exceptionally long latent period, the other a shorter latent period than that of the control strip in isotonic solution.

T A B L E I I.

Variations of the strength of the salts in relation to the latent period and to the rate and maximal height of the succeeding contractions.

Number of the experiment.	% of Na Cl	Latent Period	Height of contractions was reduced to .1 cm. in	Maximal height of contractions.	Rate when the contractions first become regular. Also the maximal rate.
35 b.	.75	38 min	50 min.	.8 cm	3 per minute--constant
36 b.	.75	24 "	70 "	.6 "	7 to 10 per min.
35 c.	1.[illegible]5	15 "	70 "	1.15 "	9.5 per minute
36 c.	1.[illegible]5	3 "	42 "	1. "	9 to 11 per minute
35 a.	.45	6 "	42 "	.7 "	5 to 8 " "
36 a.	.45	44 "	60 "	.5 "	6 to 9 " "

The [illegible] is characteristic of the saline curve of the normal strip. But if the strip is first treated with [illegible] solution or kept in terrapin's serum or blood for two or three days, or has been revived from a previous saline treatment and then again subjected to saline solution its reactions present certain characteristic differences. First and perhaps most important is the shortening or entire absence of the latent period. If the strip has already been submitted to several changes and is still in condition to contract then saline solution calls forth immediate contractions. The contractions begin at a submaximal height, usually that of the contractions in the preceding solution, quickly increase to a maximum then regularly and uniformly decrease to complete disappearance, the muscle remaining inactive in a relaxed state. In these cases the maximal sodium chloride contractions are maximal for the muscle under <u>any</u> other condition, in sharp contrast to the submaximal contractions of a fresh strip treated with sodium chloride. The rate is also much increased, in many experiments reaching 20 or more per minute, a rate too rapid to be distinguished in the records made on the slowly moving drum. The effects present the appearance of a very much heightened irritability, and the longer the muscle [illegible] the phenomena.

[illegible] sodium chloride [illegible] nearly [illegible] or perfused very much diluted blood or serum. The form [illegible] of the contractions brought out in a fresh strip by a second sodium chloride treatment is almost always perfectly regular and symmetrical. Such regularity in the response of the muscle to a definite salt in solution argues for a definite and regular series of changes in the muscle of a physical, i. e. osmotic, or chemical nature. As to the general bearing of these phenomena I prefer to leave their discussion until the effects of other solutions of the inorganic salts are presented. I shall only briefly call attention here to certain facts which are brought out by this particular group of experiments.

It must be borne in mind that the muscle strip cut from a fresh unwashed ventricle is full of blood, that is, its muscle fibers are bathed in a liquid containing the numerous constituents of blood. When this strip is suspended in a solution of sodium chloride as nearly isotonic with blood as may be, it is to be presumed that diffusion of the salts other than sodium chloride immediately begins. Presumably osmotic currents of water and the saline diffusion of sodium chloride are reduced to a minimum but all other diffusible constituents of the blood will tend to pass into the surrounding liquid. The more frequently this surrounding liquid is renewed the more rapidly the

[illegible] . [illegible] . [illegible] [illegible], [illegible] [illegible] [illegible] [illegible] blood [illegible] [illegible] few [illegible] [illegible] squeezed out [illegible] the interstices [illegible] the strip in quantities sufficient to [illegible] the saline solution.

The [illegible] before the [illegible] the [illegible] [illegible] on during irrigation. [illegible] is [illegible] more rapid [illegible] the saline solution flows through the coronary system of blood vessels. [illegible] this mechanical aid to the washing out process is most effective in irrigating the whole heart.

Recalling then, in the first place, that the apex ventricular strip cut from a heart previously irrigated and washed with saline solution will not contract when suspended in the solution, and secondly, that a heart strip filled with blood gives a rhythmic series of beats diminishing rapidly to zero, and thirdly, that a strip revived by other solutions and again surrounded by sodium chloride repeats essentially the same process, my results may be briefly summarized as follows :-

1. Sodium Chloride [illegible] [illegible]-tain the ventricle muscle [illegible] in rhythmic contractions for [illegible] of time only, [illegible].

2. The ventric[illegible] e[illegible]ted by sodium chloride solution although the decrease in amplitude of the series simulates exhaustion.

3. Sodium Chloride [illegible] a heart strip to brief series of contractions of increasing rhythm and decreasing amplitude.

4. A sodium chloride bath does not always develop maximal contractions in a heart strip.

CALCIUM CHLORIDE :

Calcium exists in the blood presumably in combination as free salts. Quantitative analyses of the calcium present in the blood of the terrapin, chrysemys picta, were made in several instances. A large terrapin was bled for such an analysis, and the blood kept on ice a day or more until the corpuscles settled. From 50 to 60 cc of clear amber colored plasma was obtained from a single terrapin. The plasma to be analyzed was siphoned off into a standardized graduated burette, measured amounts drawn into large centrifugalizing tubes and the calcium precipitated with ammonium oxalate containing an excess of ammonia. The precipitate was now left to settle or else was thrown down by means of a centrifugal machine, washed in distilled water, redissolved in weak hydrochloric acid, reprecipitated, washed [illegible] free of chlorine, dried, and finally incinerated in a platinum crucible

until the weight remained constant. Duplicate analyses were always made and the results are expressed as grams of calcium oxide in 100 cc of plasma. Each tube in experiment I, contained 24 cc., in experiment II, 25 cc. of plasma

TABLE III.

Determination of calcium in the plasma of the slider terrapin expressed as calcium oxalate in 100 cc. of plasma.

Experiment I., December 4, 1897.

a. = 0.0128 grams, b. = 0.0126 grams.

Experiment II., December 1[illegible], 1897.

a. = 0.0133 grams, b. = 0.0141 grams.

Mean of four determinations 0.0131 grams.

This determination is in close agreement with the results of Gerlach[1] made by the same method on dog's serum, .014 and .0145 grams CaO in 100 cc Serum, and also with determinations of the calcium in Sheep's serum made by Dr. Howell in this laboratory by volumetric determination with potassium permanganate, which gave as the mean of two analyses 0.0124 CaO in 100 cc of serum.

The mean of the above determinations expressed as calcium chloride is 0.026 grams per 100 cc of plasma. This amount was considered as the amount normal to the blood. Chloride salts

1. Gerlach ; Ueber die Bestimmung der Minerale des Blutserums ; Arbeiten a. d. Physiol. Anstalt zu Leipzig, p. 99, 1873.

were [illegible] order to reduce the effect of the acid radical to a constant. One would expect the amount of calcium to vary in different individuals. It is possible, too, that some of the combined calcium may separate off and if so the above amount would be too large. It will, however, serve as the most available constant in the study of the effects of the calcium in the blood in its relation to the development and maintenance of contractions in the isolated heart strip. In 1883 Ringer first demonstrated the great importance of this element in the activity of the frog's heart. Since that time numerous experimenters have extended and enlarged our knowledge of the physiological importance of calcium in the animal organism. At the present time it is generally recognized that calcium in some form plays an essential part not only in the activity of muscle but also in the clotting of blood, the coagulation of milk, etc.

Ringer demonstrated that calcium does not act alone in maintaining the rhythm of the frog's heart, but that it must be antagonized by potassium salts. I will here first discuss briefly the effects of calcium and potassium salts as such and later take up their relation to other salts. Isotonic strengths of calcium are imperfectly borne by the cardiac muscle, hence calcium effects must be studied in combination with some isotonic solution. Of these I have used isotonic solutions of sodium

chloride, however, [illegible]. [illegible]

[illegible]. The best avail-

able dextrose has generally contained traces of calcium and the re-

[illegible] therefore difficult to [illegible]. My most re-

liable calcium effects are, therefore, those obtained in combi-

nation with sodium [illegible].

CALCIUM CHLORIDE ALONE :

Calcium chloride in distilled water in approximately isotonic solution when applied directly to a heart strip throws the muscle into strong tone. No rhythmical contractions are given off for five minutes, the longest time a strip has been submitted to this excessively strong solution. When the excess of calcium is removed by washing the strip with .7 per cent. sodium chloride solution a series of very rapid contractions is immediately started. The rhythmic contractions are superimposed upon a [illegible] of [illegible] tonic shortening. No permanent injurious effect follows the use of the strong calcium solutions.

If, on the other hand, calcium chloride of the strength found in the blood is applied to a heart strip that is quiet but in good condition for contraction, [illegible] is followed immediately by a rapid [illegible]. The [illegible], the [illegible] in solu-

tion. [illegible]

[illegible] [illegible] [illegible].

[illegible] [illegible] [illegible] calcium chloride alone [illegible] [illegible]. Isotonic solu- [illegible] [illegible] calcium, [illegible] strongly [illegible] [illegible] is doubtless [illegible] more in determining [illegible].

POTASSIUM CHLORIDE.

Potassium chloride, like Calcium chloride, is deleterious when applied to the muscle of the heart if in isotonic solutions. And its effects are confused by physical phenomena when applied in solutions of a strength normally found in the blood. When [illegible] potassium chloride is applied to a heart strip which was previously [illegible] rhythmically in dilute serum the heart strip quickly [illegible] one or two spasmodic [illegible] [illegible] of tone. Afterward [illegible] [illegible] is removed by washing the [illegible] [illegible] contractions are devel- [illegible] the [illegible] spasm passes off. Dilute serum [illegible] establish- es a rhythm after a short latent period and the rhythm appears perfectly normal in character. From this it will be seen that [illegible] doses of potassium [illegible] as of calcium salts do not produce a [illegible] [illegible] strip [illegible]

at least when applied for short periods.

In more dilute solutions, i. e., in solutions approximately normal to the blood, (.03 to .04 per cent.) as determined by physiological reaction and by analyses reported for the blood of other animals,- potassium chloride applied to a contracting strip produced quiescence after a few further contractions. These contractions decrease rapidly in amplitude to complete disappearance. Tonic shortening also follows the use of this hypotonic solution.

[illegible], [illegible] AND [illegible] SALTS IN COMBINATION.

Calcium Chloride in [illegible] Solution of Sodium Chloride.

If [illegible] to [illegible] cer- tain [illegible] to [illegible]. [illegible] of salts [illegible] es a series of contractions after [illegible] latent period. The series re- sembles [illegible] features the series of contractions [illegible] The rate is more rapid in the strip in sodium [illegible] and the contrac- tions are maximal [illegible] pure sodium chloride solution [illegible] minutes after the series [illegible]. But the [illegible] characteristic effect [illegible] added [illegible] chloride solution is [illegible] each contraction. There is a [illegible] so characteristic [illegible] sodium chloride series. If the amount of calcium chloride used on a fresh strip is large, say .04 per cent. the the rise occurs very soon after [illegible] contrac- tions. [illegible] chloride treatment. [illegible] and calcium chloride [illegible] e. If the

amount [illegible] per cent. [illegible] the [illegible] is [illegible]-cular [illegible]. Calcium [illegible] chloride never more than slightly revives a heart strip after it has ceased to contract in a solution of sodium chloride alone.

CALCIUM IN ISOTONIC SOLUTIONS OF DEXTROSE.

Calcium in normal amount to the blood .028 per cent. in isotonic solutions of dextrose when applied to a heart strip previously beating in serum or in serum diluted with saline immediately calls forth a series of rapid contractions and a strong increase in tone. When the dextrose alone was applied to the heart it called forth a similar though less rapid series of contractions. It is questionable, therefore, just how much of the above effect is due to dextrose and how much to calcium.

CALCIUM IN ISOTONIC UREA.

Calcium in isotonic solutions of urea produced no contractions at all. Urea itself seems injurious to cardiac muscle when applied in isotonic strength. Even serum produces only slight or no recovery after urea.

POTASSIUM CHLORIDE IN ISOTONIC SOLUTIONS OF SODIUM CHLORIDE.

When a fresh muscle strip is immersed in a solution of .03 per cent. potassium chloride in isotonic solutions of sodium chloride either no contractions at all are developed, or if developed the contractions are extremely minute and make their appearance only after an extremely long

latent period. The contractions occurring in the exceptional cases are very irregular in their [illegible] one experiment were only one-ninth the height of the contractions given after a change to a solution of different composition. These results are in sharp contrast with the behavior of a strip surrounded by sodium chloride alone or by sodium and calcium chloride solution. Sodium and potassium chloride strips almost always show an excessive loss of tone, a result just the opposite to that of sodium and calcium strips. Sodium and potassium chloride solution has no effect in reviving activity in a strip that has ceased to beat in sodium chloride alone.

Ringer and his students have taken a prominent part in investigations concerning the action of potassium salts on the animal body and on the heart. They have shown that potassium salts applied to the frog's heart produce a slowing of the rate, much dilation, and ultimate cessation of the rhythm. Ringer was also the first to show the necessity of this salt in antagonizing the excessive stimulating effect of calcium salts. We will return to this phase of the subject.

SODIUM, CALCIUM AND POTASSIUM SALTS IN ISOTONIC SOLUTION.

The wonderful [illegible] power of sodium, [illegible], and calcium salts in solution was first pointed out by Ringer [illegible]

1883 [1]. In the pr[illegible] [illegible] it was [illegible] to reviv[illegible] and sustain the frog's [illegible] for a very long time. [illegible] phosphate for the calcium chloride but [illegible] so far as the [illegible] strip is concerned I have obtained quite satisfactory results with the chloride.

In my experiments I have striven to secure a proportion among the above inorganic salts that would give the effects on an isolated cardiac strip most nearly approaching that of blood. The facts already pointed out by Ringer and his students, and by Howell and Cook[2] seem to indicate that if one could exactly simulate the composition of the blood as regards the inorganic constituents it would be possible to secure approximately the same effects from blood and from the artificial preparation of inorganic salts in so far as the isolated strip is concerned.

By keeping the amount of calcium in isotonic sodium chloride solution constant and equal to the mean of the [illegible] of the terrapin's plasma, and by varying the amount of potassium a solution was soon determined [illegible] consistent results. With this solution results were obtained that closely approached [illegible]

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1. Ringer; The in[illegible] blood on the contr[illegible] of the heart, Journal of Physiology Vol. IV. p. [illegible], 1883. Vol. VIII. [illegible].

2. Howell and Cook. [illegible] milk, gastric [illegible] etc. Journal of [illegible] Vol. [illegible], [illegible].

effects of a serum bath on the ventricular strip. [illegible] of the three salts in this solution are :

.08% to .1% Sodium Chloride.

.026% Calcium Chloride.

.03% to .04% Potassium Chloride.

With or without a trace of sodium carbonate.

This solution of the inorganic salts found in blood will keep a heart strip alive and in condition to beat for a very long time, 72 hours and more. The inorganic solution does not keep the strip in as good condition as does blood or serum but the parallelism between the two is very striking. The following experiment exhibits the close relation in the action of the above inorganic salt solution and of serum on ventricular strips .

<u>Experiment 34, Jan. 29, 1903.</u>

The apex third of the ventricle was cut into thin strips and while still saturated with blood these were suspended in heart tubes. Strip <u>a</u> was immersed in pure serum ; strip <u>d</u> in a bath of Ringer's mixture (.8% Na Cl, .026 Ca Cl_2, .04% K Cl and a trace of $Na_2 CO_3$).

<u>a</u>. The serum strip gave a few beats when first suspended [illegible]. [illegible] contractions occurred at long and irregular intervals [illegible] 96 hours, when the strip was changed to a bath of .[illegible] per cent

sodium chloride. Sodium chloride solution called [illegible] -

[illegible] series of contractions.

d. [illegible] re-

laxed state giving only occasional [illegible]

hours. The contractions during the portion of this time that

they were recorded did not average more than one per hour. At

the end of 78 hours when the strip was changed to a bath of

.6 per cent sodium chloride a rapid series of contractions was

produced at once. While the maximal contractions of this series

were only .5 cm. high, about one half those of [illegible] in saline,

still the series was a typical sodium chloride series in every

other respect.

The following is a second example of the similarity of action

between the solution of inorganic salts and pure serum.

Terrapin 45, Feb. 23, 1898.

The apex of the ventricle saturated with blood was cut

into thin strips and suspended in heart tubes,- a in Ringer's so-

lution, b and c in air. Strip b was moistened by occasional

drops of Ringer's solution (.7% Na Cl, .02% Ca Cl_2 and .03% K Cl),

and c with drops of serum.

Strip a, after a latent period of 30 minutes gave contrac-

tions that were perfectly [illegible] irregular

in rate. [illegible] per minute.

Neither *b* nor *c* contracted for 30 consecutive hours. But when moistened with sodium chloride solution both strips gave contractions that were complete and apparently normal in character.

This again demonstrates that solutions of inorganic salts as well as serum, are able to keep the ventricular strip in good condition, and that they like serum will not necessarily stimulate the strip to rhythmic contractions.

This experiment is also important in its bearing on another experiment to be given presently, Experiment 42. The fact that one heart strip immersed in a given inorganic solution beats while another strip from the same heart does not beat when in air saturated with water vapor and moistened only occasionally with the same inorganic solution, indicates some process more favorable to the development of contractions in the presence of the liquid-bath. This can not be ascribed to any hindering effect due to the presence of the air. In fact one would expect the better aeration of the tissue surrounded by air to facilitate the development of contractions rather than to delay contractions. This point is brought forward and emphasized at this time for if strips be first washed out in sodium chloride solution then bathed for a few minutes with Ringer's solution and suspended in air they are as much as ten times more active than strips kept in a bath of the same solution.

The particular results to be expected when a fresh strip of ventricle containing the [illegible] of blood is subjected to a bath of sodium, calcium and potassium chloride solution, as far as my experiments go, depend upon the relative proportions of the calcium and potassium salts. If these salts are in the proportions of .026 per cent. calcium to .03 per cent. potassium then a few good contractions at a very slow and irregular rate result. If this ratio is changed by increasing the calcium or by decreasing the potassium then the contractions are increased in frequency. But if the calcium is diminished or the potassium increased then the contractions developed are very few or none at all. Experiment 34[d] mentioned on page   gave 15 contractions in the first 46 hours. The solution used contained .6 per cent. sodium chloride, .026 per cent. Calcium chloride, .04 per cent. potassium chloride and .003 per cent. sodium carbonate.

If the muscle strip is cut from a heart previously irrigated with saline or one that has been subjected to the influence of some other artificial solution, then the effect produced on the strip by Ringer's solution varies according to at least two factors, first the relative amounts of calcium and potassium in the Ringer's solution, and second the composition of the solution to which the strip has previously been submitted and the time it has acted.

RINGER'S SOLUTION FOLLOWING SODIUM CHLORIDE SOLUTIONS.

A ventricular strip treated with sodium chloride, .6 to .7 per cent. until it ceases to contract is stimulated to a strong and regular series of contractions when bathed in the proper solution of Ringer. The amplitude and general character of the contractions of a strip revived in this way can only be compared with the contractions of a strip revived by serum or by serum diluted with saline. By moistening the strips with Ringer's solution in which the proportion of calcium is increased from time to time, strips may be kept beating for 60 hours and more after the apparent exhaustion in sodium chloride. In one experiment, number 42, three companion strips were in this way kept contracting for 51-1/2, 62 and 42 hours, respectively.

In another experiment, 33 a. and d, strips immersed in Ringer's solution contracted rhythmically and normally for 27 hours when the record was lost. After 42 hours these strips were quiet but apparently in good condition for contraction as both strips were immediately revived and gave fine contractions when changed to serum. The recovery in both these strips in serum closely resembles the recovery of contractions in strips treated with sodium chloride and then changed to Ringer's solution or to extract of serum. This suggests a recovery due to the effects of the better [illegible]

serum. At least I am strongly inclined to believe that the revival in this case is due to the fact that serum contains the con[illegible] or [illegible] to the [illegible] of the contractile material still in the strip.

If the strip is [illegible] from sodium chloride to Ringer's solution while the contractions are small but still present, regular in rate, and similar in general character to contractions of full amplitude then the revival of complete contractions in the Ringer's solution is very prompt. The contractions become maximal in a very few minutes and the rate is often perfectly regular for a time. Afterward, however, the rate becomes slower and very irregular, a result perfectly analogous to the serum effect under the same circumstances.

On the other hand, if the contractions have entirely ceased in sodium chloride and the muscle has remained in the solution from one to several hours and then is subjected to a bath of Ringer's solution, the result is quite different. Under such circumstances the muscle is [illegible] a[illegible]tion from which it never recovers while in the Ringer's solution. This is apparently [illegible] calcium [illegible]. If, instead of the ordinary combination of salts in the Ringer's solution, a solution [illegible] o the strip and the calcium gradually increased to the normal amount

t[illegible] [illegible] co[illegible] to [illegible] [illegible] [illegible] [illegible] [illegible].

[illegible] strip [illegible] [illegible] [illegible] [illegible]

[illegible] [illegible] [illegible] ordinary Ringer's s[illegible]on, whether [illegible]. If fibrillation results then the [illegible] shortening [illegible] or even half the amount of shortening [illegible] [illegible] the same muscle [illegible] contraction. If the [illegible] strip is treated with a solution of [illegible] chloride and potassium chloride before the Ringer's solution is used the contracture is diminished and fibrillation delayed [illegible] avoided.

In certain experiments normal Ringer's solution following sodium chloride solution produced an excellent recovery for a time but later the strips went into a state of fibrillation. Perhaps it would be better to describe this state as one of inco-ordinated contractions followed by fibrillation. Some strips giving regular normal contractions in Ringer's solution, suddenly show independent rhythm in two or more parts of the strip at the same time, and this gradually passes into fibrillation.

In a [illegible] example a strip [illegible] [illegible] after repeated [illegible] from sodium chloride solution [illegible]

[illegible] [illegible] [illegible]. [illegible]

increased from .6 cm. to [illegible]. The [illegible] irregular

[illegible] av[illegible]

of .5 to 2.1 per minute. [illegible] is presumably due to the [illegible].

<u>RINGER'S SOLUTION FOLLOWING SODIUM AND POTASSIUM CHLORIDE SOLUTION</u>

A [illegible] contracting by potassium and sodium chloride [illegible] in contractions when the bath is changed to Ringer's solution.

If the amount of potassium chloride in the sodium and potassium solution has been great say .04 to .05 per cent. then the contractions called forth by the Ringer's solution may be at a very slow rate, or in groups of contractions at a good rate but the groups separated by long periods of quiet during which the muscle remains in a perfectly relaxed state. Ringer's solution after sodium and potassium chloride solution is not followed by tone shortening such as occurs after sodium chloride alone.

If a strip in a sodium and potassium chloride solution be transferred first to a Ringer's solution which has a reduced amount of calcium and later the calcium is increased, then no contractions are developed until the amount of calcium in the Ringer's solution is increased to at least the normal amount found in blood.

[illegible] strip [illegible] Ringer's solution after treatment with a solution of sodium and potassium chloride, [illegible]

[illegible]

[illegible]

case [illegible] strong [illegible] [illegible]

[illegible] [illegible] to

[illegible] [illegible] p.

EXTRACT OF THE [illegible] RESIDUE OF SERUM.

Terrapin's serum was dried over a water bath, the dried residue pulverized and extracted with distilled water. The filtered extract was evaporated to dryness and a second extract made and diluted to the original volume of serum. The second extract gave only a very faint color change when tested for proteid by the Xanthoproteic test, and no proteid was detected by Millon's reagent. Careful testing for sugar with Fehling's fluid gave a questionable trace. This extract was compared with blood and with Ringer's solution in its reviving effects on the heart strip after saline treatment. The result is given in the following experiment :

Experiment Number 46, March 2, 1898.

Three ventricular strips cut from the normal heart were first immersed in saline until the contractions produced in each were reduced to .04 mm. and less in height. Strip a. was transferred from saline to Ringer's solution (.7 NaCl, .026 Ca Cl^2, .03 K[illegible]) ; b. to serum and c. to extract of the same [illegible].

S[illegible] 1.

[illegible] as soon as [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] .4 [illegible]. [illegible] [illegible]. [illegible] [illegible] re[illegible] [illegible] 2 [illegible], [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible].

S[illegible] 2. [illegible] [illegible] [illegible] [illegible] [illegible].

S[illegible] [illegible].

[illegible]he [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] re-[illegible] of the amplitude, but not as [illegible] a re[illegible] as [illegible] of strip a. in Ringer's solution. The [illegible] [illegible] [illegible] 30 min-utes from .[illegible]3 [illegible]. [illegible] .[illegible] [illegible]. [illegible] [illegible] [illegible] [illegible] [illegible] was slightly irregular being from [illegible] [illegible] per minute. After [illegible] [illegible] [illegible] sent [illegible] [illegible] [illegible] [illegible]' [illegible] [illegible]. There were [illegible]0 [illegible] these groups in 40 minutes. In every group [illegible] [illegible] [illegible] began at a slow rate, increased to a maximum rate then became slow again. A pause of from 30 to [illegible]0 seconds intervened between each two of the several groups.

The [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible], 2 per minute, [illegible] [illegible] [illegible]. A[illegible] [illegible] [illegible] [illegible] [illegible] [illegible]

thirty [illegible].

With the exception of three [illegible] strips remained quiet in relaxation until [illegible] the customary saline series. [illegible] extract series were 1.3 cm. high. [illegible] series [illegible] very much like that of a slightly diluted [illegible].

On the whole [illegible] were normally, that is more like serum, than [illegible] Ringer's [illegible] in the case of Strip a. The after effects were [illegible] more like [illegible] as shown in other serum experiments. Another experiment with serum extract bears out this view. Serum extract was used on a strip in fibrillation after long exhaustion. It at once produced increased activity in the fibrillating strip which after 70 minutes suddenly relaxed and began slow and irregular yet apparently perfect contractions. The relaxation was however only partial in this case. This was the only time in all my experiments that I obtained this type of recovery with other than pure serum.

ON [illegible] THE SOURCE OF CONTRACTILE [illegible].

The amount of [illegible] in an inorganic salt [illegible] experiments as to raise the question as to the [illegible] of [illegible]-

giving material. In experiment number 33 a. a fresh strip that had been bathed in .[illegible] per cent. sodium chloride until no further beats were produced, was changed to a bath of Ringer's solution. It almost immediately gave contractions which were normal in general character and higher and stronger than the largest contractions obtained from the strip while in the sodium chloride bath. The rate, however, was slow and irregular.

The contractions of this strip in Ringer's solution increased in height and improved in rate and in 27 hours gave off 1670 gramcentimeters of work. The record was lost from the 27th hour till the 42nd hour at the end of which time the quiet muscle was transferred to serum. This partial record strongly suggests the view that the amount of energy developed by the muscle strip is greater than can be accounted for by the proteid constituents of the blood in the muscle. To test this question more thoroughly, Experiment number 42, given in detail below was tried.

In this experiment the total series of contractions of each one of the strips while moist with Ringer's solution was counted the average height for each hour measured, and the total work done computed in gramcentimeters.

Experiment 42, February 11, 1898.

A terrapin's heart was washed as free as possible of blood by continuous irrigation with .7% sodium chloride solution. The washing was facilitated by cutting the coronary veins and by gentle massage applied to the ventricle. Irrigation continued one hour and at the end of that time the ventricle was still giving feeble contractions. Thirty minutes were consumed in preparing, weighing and suspending three ventricular strips. They were, therefore, subjected to sodium chloride 1 hour, 30 minutes. The strips in the heart tubes were next surrounded by a bath of Ringer's solution (.7% Na Cl, .026% Ca Cl^2, and .03% K Cl) for thirty minutes, during this time strong contractions were established in each strip. The Ringer's solution was next drawn off leaving the strips in moist air. They were occasionally moistened with a momentary bath or with drops of Ringer's solution. The three strips were kept thus in air 51 1/2, 72-2/3 and 50 hours respectively.

Strip a.

This strip was kept moistened with Ringer's solution, of the composition given above, for 17 hours, 25 minutes. The contractions were comparatively rapid at first, about one-half the total number of contractions of the strip occurring during the first seven hours. During this first period of suspension

[illegible], [illegible]

was beati[illegible] [illegible] [illegible] groups. The rate temporarily [illegible] [illegible] remoistening of the strip. The amplitude [illegible] .9 cm [illegible] shortening during a contraction, slowly but [illegible] increased to 1.35 cm. in twenty-[illegible] hours [illegible] [illegible]. [illegible] [illegible]. The contractions were apparent[illegible] [illegible] [illegible] rate was fast or slow. The record was lost from the 10-1/2th hour to the 1[illegible]-1/2[illegible] hour of suspension in air. At the end of this time strip [illegible] [illegible] [illegible] [illegible]. [illegible] with the usual Ringer's solution produced no contractions. Ringer's solution with increased amounts of Calcium chloride was [illegible] applied to the strip. During eight hours (17th to 25th hour) the calcium chloride was increased successively to .0[illegible], .04[illegible], .0[illegible] and .0[illegible]. [illegible] Ringer's solution [illegible] .0[illegible] [illegible] chloride [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] twenty-six hours thirty [illegible], [illegible] 5 [illegible] [illegible]. The contractions [illegible] were [illegible] [illegible] [illegible] increase [illegible] [illegible] ([illegible] experiment) [illegible] ([illegible]). [illegible] [illegible] [illegible] [illegible] 0° C. [illegible] [illegible], but [illegible] recovered [illegible].

The [illegible] hour after [illegible] contractions be-
to slowly decrease in amplitude reaching zero in six hours thir-ty minutes, i. e. after a total time of activity in Ringer's solution of [illegible]1 hours and 30 minutes. The proportion of calcium was again increased but no contractions were produced even with 0.1 per cent. calcium chloride.

A bath of horse's serum applied to the strip at 51 1/2 hours was followed immediately by a series of contractions lasting for 16 hours. The rate began with 5 per minute, but varied greatly at different times and the contractions were often quite uncoordinated. The height in serum was .35 cm at first, about one-third that of the normal contractions of this strip but decreased to zero in 16 hours, i. e. the 67th hour of the experiment. Minute contractions, .02 cm. were obtained by diluted serum at the 74th hour.

Strip b.

This strip was continued in air for 71 hours. For the first 10 hours the record had all the general features of a. except that the contractions were at a somewhat slower and more irregular rate and were higher. Luciani's groups with well-marked [illegible] occurred after 9 hours and until the 22nd hour. Only 16 contractions occurred from the 20th to the 22nd hour of suspension in air. Ringer's solution with the calcium increased to .04 % was used to wet the strip at the 2[illegible] hour and was imme-

iately followed by tall regular contractions for over two hours. These contractions ceased quite abruptly and after a long pause the strip was wet with drops of Ringer's solution containing .05% calcium, the 2[illegible] hour. Immediately Luciani's groups with long intervening pauses appeared. The groups became more and more frequent and after 3 hours passed into an irregular rhythm which continued through 35 hours 30 minutes. During the night the rate and height were both decreased by the low temperature as in a. The calcium chloride was again increased in the Ringer's solution to .08% and at 51 hours, 30 minutes the strip was given a two minutes bath. The only effect was a slight increase in rate.

Following the 50th hour of suspension the height of the contractions slowly decreased for 12 hours from .98 cm to zero. Bathing the strip with Ringer's solution containing .06% and .08% calcium chloride brought out contractions of good rate but only a millimeter in height, not recorded in the table below). At 72 hours strong electrical stimulation produced no observable contractions.

At 72 hours, 40 minutes the strip was immersed in a bath of horse's serum which produced contractions only .02 cm. to .03 cm. in height, i. e. no recovery. The serum in this case, therefore, failed to cause an improvement in contractions. The experiment ended at the 74th hour after suspension in air.

Strip c.

This strip in air contracted rapidly for a time but gradually became slower during 24 hours and 45 minutes. Its contractions decreased in rate more gradually than in a or b and Luciani's groups were not so prominent. Wetting the strip with Ringer's solution with Calcium chloride .04 per cent at 24 hours 45 minutes was followed by an increase in the rate. After 30 hours 15 minutes increase of calcium chloride to .05 per cent produced a slight increase in the rate.

The height of the contractions slowly decreased from .39 cm. at the 31st hour to zero at the 42nd hour (see table below). The number of contractions for the last ten hours was very small. Further increase of calcium produced in each case comparatively rapid contractions, but no recovery of the height ; i. e. 42nd hour, calcium chloride .06 per cent height of the contractions immediately following .14 cm ; 44th hour .08 per cent calcium chloride, .18 cm ; 46th hour, .10 per cent. calcium chloride minute contractions.

At the 50th hour the strip was given a bath of terrapin's serum which produced contractions only .15 cm high, but no strong full contractions resulted. I have made the amount of work given by these three strips of ventricular muscle contracting automatically in a purely inorganic diet the basis of a series of calculations, in order to express the results in a way that admits of comparative study.

FOLD OUT

... done during
... our ... press-
as gramcenti-
ers.

	[illegible].		c.
1	396	gram	440
8	371	"	430
2	294	"	330
4	249		292
3	173	"	293
6	123	"	305
	1		
5	4958		4061

5000 Cal. etc.
Rubner Vol XX
Vol 30
[illegible] for [illegible],

5700

The strips of muscle were weighed as carefully as possible before suspending them, also at the close of the treatment with inorganic salts. At each weighing the moisture was rem ved to as nearly the same extent as was possible by draining on glazed porcelain or on glass. They were then weighed between watch crystals. The results of all experiments where double weighings were made show a loss of weight. This fact is of signi icance although I have not made a sufficient number of experiments for quantitative estimates from this standpoint.

TABLE V.

Weight of muscle strips at the beginning and end of treatment with Ringer's solution :

Number of the Experiment	Weight before treatment with Ringer's solution.	Weight after treatment with Ringer's solution.	Time of suspension in Ringer's solution.	Loss
42 a.	0.321 grms.	0.196 grms.	51-1/2 hours	0.125 grms.
42 b.	0.283 "	0.231 "	72 "	0.052 "
42 c.	0.340 "	0.232 "	50 "	0.108 "

The figures in [illegible] the weights before the use of Ringer's solution are of the greatest importance for the present consideration. The three strips used gave respectively 4915, 4953, and 4631 gramcentimeters of motorial work. If this work be converted into its heat equivalent it may then be compared with the heat equivalents of the possible sources of energy-giving material. One gramcentimeter of work equals 980 ergs ; one calorie equals 4.2×10^7 ergs ; one gramcentimeter therefore equals 0.000024 calories.

The total heat of oxidation of one gram of proteid or of cane sugar varies according to the determinations of different investigators. Stohmann[I] gives for fibrin 5503, [illegible] 5577, for urea 2465, and for cane sugar 3960 calories respectively. Danilewsky[2] gives for peptone 4376, fibrin [illegible], urea 2537, and for cane sugar 4176 calories. If the oxidation equivalent of the urea formed as a result of the metabolism of one gram of proteid is deducted then it may be assumed that in round numbers the average oxidation energy of one gram of proteid or one gram of sugar available for muscular metabolism [illegible] or 4 [illegible] calories for carbohydrate.

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1. Stohmann, Jour. f. prakt. Chem. [illegible], p. [illegible], [illegible] Landwirtschriftl. Jahr. [illegible] p. [illegible] (quoted [illegible] Phys. Chemie)

2. Danilewsky, Ueber die Kraftvorräthe der Nahrungsstoffe, Pflüger's Archiv, Vol. 3[illegible], p. [illegible], [illegible].

[illegible] amount of work recorded [illegible] the [illegible] is the equivalent of the oxidation of [illegible]0025[illegible], .[illegible]002[illegible]7, and .[illegible]0021[illegible] grams of proteid or of .[illegible]0002[illegible], .[illegible], .00002[illegible] [illegible] of carbohydrate respectively. These amounts represent the oxidation equivalents if all the energy appears as work, whereas it is well known that only a small fraction of the total energy of metabolism can be utilized as work. Gaule [1] 187[illegible] computed that at least not less than 8[illegible] of the energy of contraction in the frog's heart may be recorded as work. The well known experiments of Fick upon striated muscles show that under the most favorable conditions 20 per cent. may be regarded as a maximum yield in work. In the cardiac strip, it must be remembered that many of the fibers are cut across so that their contractions are lost, while others may by their contractions oppose those which exert [illegible] direct pull on the lever. It is, therefore, a [illegible] estimate to [illegible] that 15 per cent. of the energy of oxidation may take the form of work during contractions of the ventricular strip, and that not more than two thirds of this, 10 per cent of the total energy, is recovered on the record. These facts are arranged for comparison in the following table :

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1. Gaule; Arch. [illegible] . F[illegible] . 310 [illegible] .

TABLE VI.

Number of the experiment.	Work recorded.	Mechanical equivalent in heat.	Total oxidation equivalent in protoid.	Equivalent in protoid of metabolism if 10% is recovered as work.
42 a.	49[illegible] grm	.114[illegible] Calories	.[illegible] grms.	.[illegible]0255 grms.
42 b.	4956 "	.11569 "	.0000257 "	.000257 "
42 c.	3991 "	.09312 "	.0000211 "	.000211 "

The average amount of serum albumin in the serum of this species of terrapin during the winter season as determined by Howell [1] in 1884 is .69%. If the above energy is supplied by the metabolism of serum albumin as Kronecker holds then the amount of blood each of the three strips above must have contained in order to supply the necessary albumin is [illegible]%, 13.1% and 9.[illegible]% respectively of their initial weights. But the percentage of blood in the entire body (determined upon mammals) is only 7.7 %, a large proportion of which is contained in the great vessels. The percentages exhibited in the following table reduce the question to an absurdity in so far as considering serum albumin as the source of the motive energy of the terrapin's heart is concerned.

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1. Howell; Composition of the blood and lymph of the slider terrapin. Studies from the Biological Laboratory of the Johns Hopkins University p. 49, 1884.

T A [illegible] VII.

Number of the experiment.	Work recorded	Equivalent in the metabolism of serum albumin.	Weight of serum necessary to supply the serum albumin.	Initial weight of the muscles.	Proportion of serum in the muscles necessary to account for the work etc.
42 a.	4915 grams	.000255 grms.	.03[illegible] grms.	.3[illegible] grms	[illegible].5%
42 b.	4958 "	.000257 "	.037 "	.333 "	13.1%
42 c.	4051 "	.000211 "	.031 "	.340 "	9.0%

The amount of paraglobulin in the blood of this terrapin is, according to Howell, comparatively large, 4.63 grams per 100 c.c. for the sake of the argument the equivalent of the above work may be expressed in terms of paraglobulin.

T A B L E VIII.

Number of the experiment.	Work recorded	Equivalent in the metabolism of paraglobulin.	Weight of serum necessary to supply the paraglobulin	Initial weight of the muscles.	Proportion of serum in the muscle strips necessary to supply the paraglobulin
42 a.	4915 grams.	.000255 grms	.0055grms	.321 grms	[illegible].7%
42 b.	4958 "	.000257 "	.055 "	.333 "	1.9%
42 c.	[illegible] "	.[illegible] "	.[illegible] "	.[illegible] "	.8[illegible]

When it is remembered [illegible] .7 sodium chloride for one hour [illegible] that the [illegible]

by the contractions of the [illegible] during the time, then the possibility of there being [illegible] large percentage of blood left in the capillaries or in the [illegible] of the tissues seems improbable.

The percentage of sugar in systemic blood [I] varies from .1 per cent to .15 per cent. On this basis it would be utterly impossible to account for the work, assuming that the energy came from the consumption of sugar furnished by the serum still left in the strips.

The more rational view applicable to this case, is that the heart has stored material in its cells and that the contractions of the isolated ventricular strips are at the expense of this material. The voluntary muscles are admittedly able to contract at the expense of stored contractile material. Why, therefore, should this function be denied to cardiac muscle ?

The above experiment seems to demonstrate beyond doubt <u>that the terrapin's heart contracts at the expense of an antecedent contractile substance stored up in its own tissue</u>.

The amount of work developed by the heart strip under the influence of sodium chloride solution or in fact any of the inorganic solutions used is perfectly represented by the [illegible] of

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I. Seegen, Biol. Centralb. p. [illegible], [illegible].

the hei[illegible] and rate of contractions [illegible] various headings, since the load was kept uniformly at one gram with all the levers used in the experiments quoted.

EXHAUSTION OF HEART MUSCLE.

The word "exhaustion" as applied to the heart [illegible] used to express many different states. As [illegible] longer use the term without specifying the condition to which it applies. Before defining its use in this chapter it will be interesting to briefly sketch its use in the literature on the subject.

In 1874 Kronecker and Stirling found that a frog's heart filled with .6% sodium chloride solution soon ceased to beat and could be made to beat again only on the introduction of pure or dilute serum. They considered this condition brought on by sodium chloride solution as one of exhaustion. From these experiments they were led to the conclusion that the heart in its contractions used material obtained directly from the blood. According to the view proposed by Kronecker [illegible] the time the frog's heart is exhausted in sodium chloride solution because the material of the blood necessary to carry on contraction is washed out.

...vestigations tending to support ... Kronecker's views are in chronological order, ... 1879, Martius 1882, Kronecker and Popoff 1887 and White 1887.

In 1878 Gaule showed that a heart ... in ... sodium chloride could be made to contract for several ... by ... a solution of alkali-saline, and that in this latter solution it gave off as many as one thousand contractions. This remarkable result can be explained, he says, only on the supposition that the muscle of the heart has the antecedent material which it uses in contractions stored up in its substance. When a heart will no longer beat in changes of alkaline sodium chloride solution, according to Gaule, it can be revived only by feeding it with blood. Apparently, according to his view, a heart is exhausted when its store of contractile material is used up.

In 1882 Martius working under the direction of Kronecker ascribed the beneficial effect of alkali-saline not to a more complete utilization of stored material in the heart cells, as Gaule supposed, but to the fact that the alkali by combining with carbon dioxide prevented asphyxiation of the contractile tissue ... permitted a more complete utilization of ... still in the interstices of the heart. When this material is used up then, and only then, ... Martius

stated that [illegible] heart [illegible] sub-stances like blood, serum, lymph, i. e. [illegible] se-rum albumin, and he concluded th[illegible] was the partic-ular element in the blood used by the cardiac muscle in contrac-tion.

In 1883 and 1885 Ringer gave a new interpretation to the term exhaustion when he said "calcium salts are necessary for the proper contractions of the heart yet they must be antagonized by potassium salts." According to this idea a heart fails to con-tract in sodium chloride or in alkali-saline because it does not have supplied to it the necessary calcium and potassium salts.

Ringer's standpoint was still further emphasized by Howell and Cook in 1894, who showed that hearts that had ceased to beat after abundant irrigation with saline or alkali-saline could be revived and kept in normal contractions for long periods when supplied with Ringer's solution or with extracts of milk, blood, or gastric juice that contained only traces of proteid.

When in 1[illegible]7 White again wrote in support of Kronecker's view he was compelled to take refuge in a hypothesis [illegible] the most pains-taking work could be overthrown by one word. This hypothe-sis assumed that the heart may beat on infinitesimal and, one may add, indetectable quanti[illegible] -

most if not quite impossible to completely remove all traces of serum-albumin from the muscular spaces of the heart. White said that by the methods used by Merunowicz, Albert, Martin and other investigators "it was impossible to completely wash out a heart." He held, therefore, that their conclusions were unfounded. He said that a heart must be irrigated through the most improved perfusion cannula, that it must be irrigated by successive solutions of sodium chloride, alkali-sodium chloride, and Ringer's solution until it is quiet. When it no longer responds to any of these solutions then it is free from serum-albumin and is truly exhausted. This is however, the familiar argument in a circle since the conclusion proceeds directly from the hypothesis assumed in the beginning. White also committed the grave error of not using the most favorable combination of salts in Ringer's solution and, therefore, did not secure completely washed out hearts in the sense in which he uses the term. By his own process of reasoning his results must be placed in the same category as those of the earlier investigators. In the examples that he gives the hearts were [illegible] only after a total time of irrigation of :

1 hour 25 minutes,

[illegible] hours 30 "

9 " [illegible] "

4 " [illegible] "

In this laboratory in experimenting upon the whole heart of the frog, the results of which will be published later, it has been observed that after the heart has ceased to beat upon the Ringer's mixture used by White it may still beat and beat well upon the mixture generally employed in this paper (.7% Na Cl, .026% Ca Cl^2, and .03% KCl). Moreover, with many hearts, although not with all, it has happened that after they have ceased to beat upon this last mixture the gradual increase of the amount of calcium salts in the Ringer's solution called forth new beats for a considerable period, an effect also obtained on heart strips as described in this paper.

From the above review it will be seen that the term "exhaustion" as applied to the heart either expresses states in which the inorganic salts necessary to the contractions of the heart are removed or disturbed in their relations, or it expresses states in which the antecedent organic contractile material is consumed or removed.

In the first group may be included as many conditions of exhaustion as there are combinations of the inorganic salts that will not support contractions. That the series of beats obtained with any solution should disappear in successively smaller contractions, as they do in a heart [illegible] with sodium chloride solution, is non-essential. In the second group must be included

on the one hand Kronecker's view that exhaustion signifies a lack of sufficient serum albumin to support contractions, and on the other Gaskell's view that exhaustion means a lack of stored contractile material.

It seems to me it would be better to restrict the application of the term to states of the cardiac tissue itself and to designate in some other way all those conditions which imply the presence or absence of some substance or substances in the surrounding blood or artificial fluid. My own experiments are full of examples that demonstrate the inefficiency of sodium chloride in producing a true exhaustion in the ventricular strip. Time after time the sodium chloride solution series has been almost exactly duplicated on the same strip after an intervening recovery due to a bath of solutions of inorganic salts alone.

It is admitted that an inorganic diet can not serve directly as a source of energy. My experiments give no convincing proof of the ability of the isolated muscle to use even organic material from serum.

In experiment Number 42 given above, strips a. b. and c. were made to contract incompletely with serum after they could no longer be aroused by Ringer's solution, but b gave only the slightest movements when treated with serum. In both a. and c. the revival of contractions due to serum treatment did not last as long as

[illegible] the contractions in b. [illegible] it [illegible]ation. It is possible, [illegible], that the [illegible] effect on a. and b. was [illegible] [illegible] ation of the inorganic salts and was only a more perfect exhaustion of the contractile material stored in the muscle cells of the strips.

The particular type of dying out of the contractions given by the strips quoted in experiment 49 above, in which after many hours the amplitude of contractions slowly but steadily decrease to a zero from which no good recovery can be obtained, has occurred so often that it may be expected with confidence whenever muscular strips of ventricle are treated with any solution favorable to the development of continuous rhythmic contractions. In fact in the very first experiment of this investigation a heart strip after 72 hours continuous contraction ceased in the same way as a. and b. above. This particular strip was suspended in a muscle moist-chamber and was made to contract by moistening it with serum much diluted with .6% sodium chloride.

This type of dying out of the contractions I take to indicate a using up of the organic contractile material in the muscle. The completeness of exhaustion in this sense depends upon whether the muscular strip is subjected to the most favorable relation of the inorganic salts of the blood, especially of the sodium potassium and calcium salts. Whether [illegible] isolated strip is capable of being nourished that is of [illegible] stored energy of the

ormed in constituents of the liquid in which it is immersed is an independent question.

SUMMARY :

1. Normal serum will not keep the ventricular apex strip in contraction, although it keeps it in good condition for contraction for three or four days. By slightly increasing the amount of calcium in the serum regular contractions may be produced.

2. An artificial mixture of sodium, potassium and calcium salts in the proportions in which they exist in serum acts like serum in that it does not produce a continuous series of rhythmic contractions but sustains the cardiac strip in good condition for contraction for at least three days. For the terrapin's heart this proportion is approximately .7 per cent. sodium chloride, .026 per cent. calcium chloride, and .03 per cent. potassium chloride.

3. Sodium chloride will produce and sustain contractions for a short time only, and the series of contractions presents the appearance of fatigue. This appearance of fatigue indicates only the removal of the inorganic salts necessary for contraction and is not an exhaustion of the contractile substance of the muscle.

4. Calcium salts in isotonic solutions of sodium chloride

stimulate the cardiac strip to increase rhythm and final permanent contracture.

5. Potash in isotonic solutions of sodium chloride prevents contractions and keeps the ventricular strip in a state of relaxation.

6. There is an optimum ratio of the potash, calcium, and sodium salts in isotonic solution most favorable to the development and maintenance of the contractions in the ventricular strip. For the apex of the ventricle of the terrapin (Chrysemys picta), this proportion is sodium chloride .7%, calcium chloride .04% or .05%, potassium chloride .03% , if the strip is fresh and filled with blood. If the strip is from a heart first washed with .7% sodium chloride the proportion given in number 2 above is the most favorable. The rhythm in the spongy ventricular strip is rarely perfectly regular in this solution.

7. Complete exhaustion of the contractile substance in the heart of the winter terrapin is brought about by the use of inorganic salt solution only after 30 to 72 hours continuous rhythmic activity, or by 72 to 100 hours suspension if the activity has been slight.

8. Cane sugar and urea, in isotonic solutions do not produce rhythmic contractions in the isolated strip. Dextrose in isotonic solution throws the strip into [illegible] [illegible] may produce an imperfect series of contractions.

B I O G R A P H Y.

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